Host-pathogen interaction between *Staphylococcus aureus* and murine macrophages

Chapter 1: Introduction

Staphylococci are gram positive rotund bacteria that grow in clusters; and hence get their name. The genus of *Staphylococcus* comprises of over 30 species of which *S. epidermidis* and *S.aureus* are significant in their interaction with humans and are known to cause diseases. *S.aureus* invades various soft tissues and causes a vast multitude of diseases spanning from simple boils and abscesses to osteomyelitis and endocarditis, which can become fatal upon the onset of bacteremia and toxic shock. *S. aureus* has also been established as one of the leading causes of nosocomial infections especially because of their multi-drug resistant traits and their ability to colonize prosthetic devices and catheters. The increasing incidence of the multi-drug resistant strains and the rising prevalence of community acquired *S. aureus* infections mandates a comprehensive understanding of the pathogen and its biology, its intracellular fate and the defense mechanisms in the host. Towards this end, we have attempted to delineate some aspects of the pathogen's virulence and the host responses to them.

S. aureus normally inhabits the skin and mucosal surfaces as a commensal. Upon the onset of permissive circumstances it turns into an opportunistic pathogen. Immuno-compromised conditions or breach of skin can serve as the portals of entry for the pathogen. Upon entry, the bacteria encounter macrophages as the first line of defense in the host. Macrophages appear at the site of infection and phagocytose the bacteria, subjecting the pathogen to phagolysosomal degradation which facilitates antigen presentation and pathogen clearance. As part of their immune evasion mechanism, various pathogens are known to adopt a multitude of strategies to subvert this fate and survive in the host cells. This dissertation work aims at gaining insight into the

staphylococcus-macrophage interaction in the ongoing host-pathogen duel, to gain better understanding about the pathophysiology and etiology of the disease.

Chapter 2: Intracellular Trafficking of *Staphylococcus aureus* in Macrophages

Successful targeting of the pathogen necessitates a comprehensive understanding of its biology and physiology in its interactions with the host. With this objective we undertook a study to uncover the intracellular niche of *S. aureus* in RAW264.7 murine macrophage-like cells. Any invading pathogen once internalized by the macrophage is contained in a phagosome, which undergoes progressive acidification and maturation from the early endosome to late endosome and ultimately fuses with the phagolysosome, where where the invading pathogen is subject to degradation. Through exhaustive electron microscopy of the infected macrophages, we show that *S. aureus* is present as a single bacterium per vacuole through the entire period of infection. We have further monitored the intracellular trafficking of the bacteria in the macrophage through confocal studies with endosomal markers which serve as indicators of vesicle maturation. Soon after the onset of the infection, the bacteria were found to be present in the early endosome (EEA-1 positive vesicles) which gradually matured into LAMP1 positive, late endosomal vesicles. However, only a small fraction of the bacteria containing vesicles were found to fuse with the lysosomes, suggesting that the bacteria prevented phagolysosomal fusion. We further observed that the bacteria did not prevent the acidification of the vesicles they resided in, but only limited their fusion with the lysosome. Taken together, our studies delineating the intracellular niche of *S. aureus* in RAW macrophages revealed that the pathogen has successfully evolved immune evasion mechanisms to overcome its phagolysosomal relegation.

Chapter 3: *Staphylococcus aureus* Succumbs to the Hepcidin in Murine Macrophage

We have further attempted to study the intracellular fate of the bacteria in macrophages towards gaining greater insight into its biology. Our studies on the intracellular fate of *S. aureus* in RAW264.7 cells revealed a distinct biphasic fate of the bacteria. The pathogen was found to replicate initially and this proliferative phase was subsequently followed by a gradual fall in its numbers. Interestingly however, the pathogen is never found to be cleared from the system suggesting the presence of a residual infective pool in the macrophages. We thus explored the possible mechanisms which could attribute to this biphasic intracellular fate of the bacteria. Macrophages come armed with a rich repertoire of defense mechanisms to incapacitate the invading pathogens. They have in their arsenal, reactive oxygen (ROS) and nitrogen species (RNS) and many potent anti-microbial peptides, apart from the lysosomal machinery, to degrade the invading pathogen. Upon investigation, we find that the RAW macrophages do not mount a ROS/RNS response when infected with *S. aureus*. Induction of these responses in the macrophage by alternate means further reveals that the pathogen is recalcitrant to death by these oxidative/nitrosative bursts. Of the antimicrobial peptides (AMPs) harbored by macrophages, we find that Hepcidin is up-regulated upon infection with *S. aureus*. Hepcidin is a peptide which is known to have a key regulatory role in iron homeostasis in addition to its potent antimicrobial functions. Since Hepcidin is known to be induced upon increased iron availability; we pre-treated the host cells with iron and monitored the effect of the same on bacterial fate. As expected, we observed that Hepcidin induction by pre-treatment with iron equips the macrophage to counter the pathogen better and thus leads to hastened and heightened clearance of the bacteria. This induction of hepcidin is significant at the mRNA and protein levels and is also

corroborated by increased co-localisation of the bacteria with the anti-microbial peptide. Our studies thus identify hepcidin as a key line of the host defense towards countering the bacterial infection thus explaining the near complete bacterial clearance observed.

Chapter 4: Global gene expression studies offering insight into potential immune evasion strategies of *S.aureus* in countering host offences.

The interactions between host and the pathogen are multi-layered with the involvement of numerous players and many signaling cascades. In this light, we have attempted to get a holistic view of the host-pathogen interplay through microarray studies. These global profiling studies were aimed at identifying the important players in bacterial virulence and the macrophage response factors involved in countering the same in the context of *S. aureus* infection. The array was uniquely designed to incorporate both bacterial and host probes so as to facilitate parallel analysis of the host and pathogen gene expression profiles in the same sample. The expression profiling studies were carried out at three time points which represent the key phases of the bacterial infection viz. internalization, replication and clearance. A comprehensive analysis of the bacterial and host gene expression profiles under these phases provided insights into bacterial virulence and the host's strategies to counter the same.

We observe a large scale metabolic shut down in *S. aureus* subsequent to its internalization. We find the distinct up-regulation of a small subset of genes, majority of which are as yet uncharacterized. Amongst these were a few well-characterized virulence genes which remained active, representing the bacterial strategies to subvert the host immune response. The large scale down-regulation of gene expression can be possibly explained as the adaptation of the bacteria to the available metabolites and its submission to a quiescent phase of existence in the macrophage. In parallel, the host

system exhibits the induction of TNF-α and up regulation of TLR2 and Nod2, which are typically triggered by a gram-positive infection. But simultaneously, we also observed a marked increase in the expression of anti-apoptotic and anti-inflammatory responses. This was re-iterated by a significant down-regulation in some of the pro-inflammatory, pro-apoptotic and antigen presentation involved genes and processes. We further find that the time course of the infection did not largely influence the gene expression kinetics. The macrophages were influenced and committed to a fate conducive for the bacteria fairly early in the infection regime. Thus, our studies of the expression profiles of the pathogen and the host under the different phases of the infection provide us with a comprehensive understanding the strategies of bacterial offense and host defenses thereby offering a window into this fascinating world of host-pathogen interactions.

Chapter 5: Conclusion

To summarize, we have attempted to study the intracellular fate of the *S. aureus* pathogen in macrophages. Our studies suggest that the bacterium attempts to evade clearance by the host immune system by actively preventing fusion with the lysosomal vesicles. We also find that despite these defenses, the pathogen appears to succumb to the host immune system as it is targeted by Hepcidin, an anti-microbial peptide. The lack of complete bacterial clearance under these conditions is however suggestive of an underlying strategy by the pathogen, possibly to maintain a chronic infective state in the host system. The microarray studies, in addition, shed light on the other possible immune evasion strategies that *S.aureus* might be employing to escape the host offences. The results are indicative of the bacteria influencing anti-apoptotic, anti-inflammatory and antigen presentation responses and thereby prolonging its survival in the macrophage.

In conclusion, given the fact that the macrophages are itinerant cells with a long life span, the light thrown by our findings of the various immune evasion strategies that *S.aureus* is adopting; it suggests that the macrophages could serve as potential carriers which could account for the dissemination of the infection to new sites, which has perpetually been a major concern for any Staphylococcal infection.